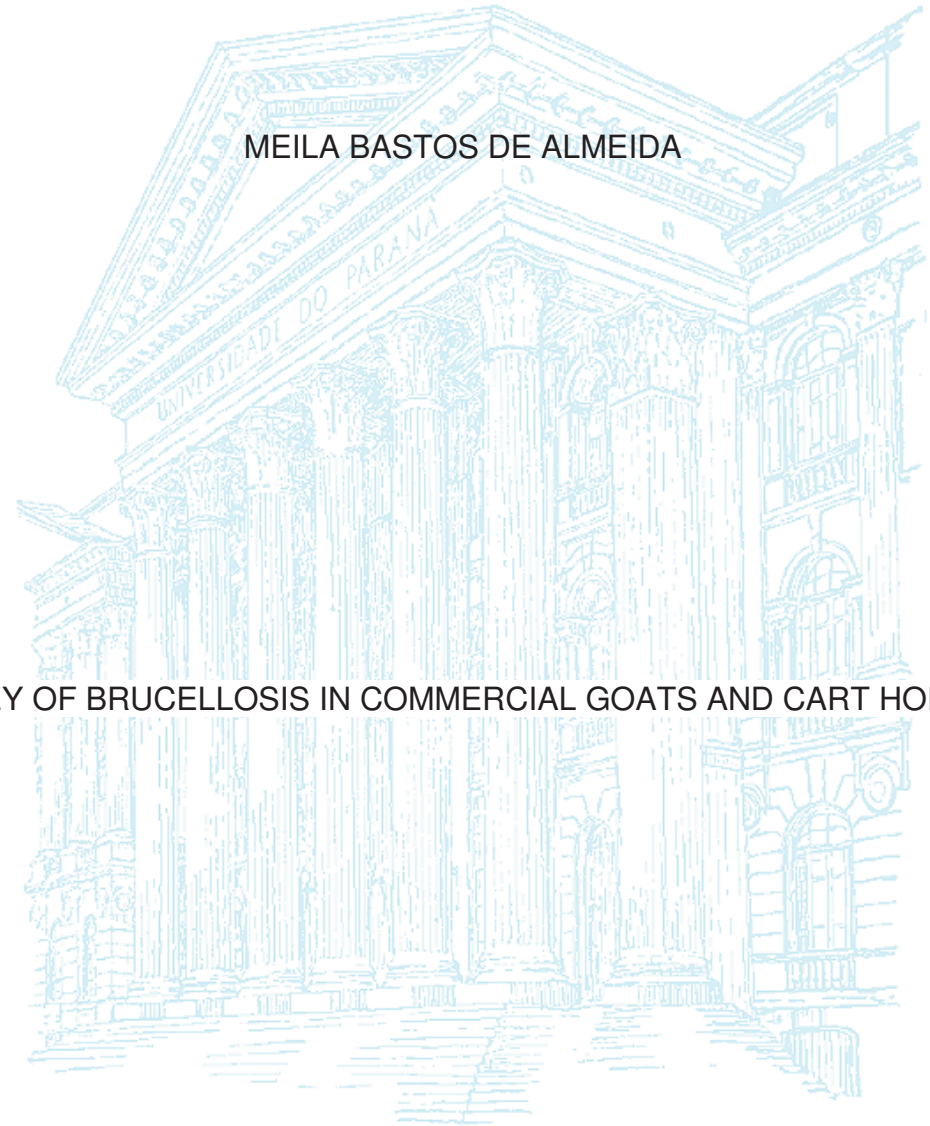
UNIVERSIDADE FEDERAL DO PARANÁ

MEILA BASTOS DE ALMEIDA



SEROSURVEY OF BRUCELLOSIS IN COMMERCIAL GOATS AND CART HORSES

CURITIBA

2018

MEILA BASTOS DE ALMEIDA

SEROSURVEY OF BRUCELLOSIS IN COMMERCIAL GOATS AND CART HORSES

Dissertação apresentada ao Programa de Pós-Graduação em Ciências Veterinárias, Área de Concentração em Saúde Única, Setor de Ciências Agrárias, Universidade Federal do Paraná, como requisito parcial à obtenção do título de Mestre em Ciências Veterinárias.

Orientador: Prof. Dr. Alexander Welker Biondo
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CURITIBA
2018

AL447s

Almeida, Meila Bastos de
Serosurvey of brucellosis in commercial goats and cart horses / Meila Bastos de Almeida. - Curitiba, 2018.
53 f.: il., grafs., tabs.

Dissertação (Mestrado) - Universidade Federal do Paraná. Setor de Ciências Agrárias, Programa de Pós-Graduação em Ciências Veterinárias.
Orientador: Alexander Welker Biondo

1. Brucelose. 2. Brucelose em equino. 3. Brucelose em caprino. 4. Brucelose em bovino. I. Biondo, Alexander Welker. II. Título. III. Universidade Federal do Paraná.

CDU 616.981.42

Sistema de Bibliotecas/UFPR, Biblioteca de Ciências Agrárias
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TERMO DE APROVAÇÃO

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em CIÊNCIAS VETERINÁRIAS da Universidade Federal do Paraná foram convocados para realizar a arguição da dissertação de Mestrado de **MEILA BASTOS DE ALMEIDA** intitulada: **SEROSURVEY OF BRUCELOSIS IN COMMERCIAL GOATS AND CARTHOSES** , após terem inquirido a aluna e realizado a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.
A outorga do título de mestre está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

CURITIBA, 01 de Fevereiro de 2018.

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Presidente da Banca Examinadora (UFPR)

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ATA Nº 5

ATA DE SESSÃO PÚBLICA DE DEFESA DISSERTAÇÃO PARA OBTENÇÃO DO GRAU DE MESTRE EM CIÊNCIAS VETERINÁRIAS.

No dia um de fevereiro de dois mil e dezoito às 09 horas, na sala Anfiteatro,do Hospital Veterinário do Setor de SETOR DE CIENCIAS AGRARIAS da Universidade Federal do Paraná, foram instalados os trabalhos de arguição da Mestranda MEILA BASTOS DE ALMEIDA para a Defesa Pública de sua Dissertação de Mestrado intitulada: **SEROSURVEY OF BRUCELOSIS IN COMMERCIAL GOATS AND CARTHOSES**. A Banca Examinadora, designada pelo Colegiado do Programa de PósGraduação em CIÊNCIAS VETERINÁRIAS da Universidade Federal do Paraná, foi constituída pelos seguintes Membros: ALEXANDER WELKER BIONDO(UFPR),JOAO HENRIQUE PEROTTA(UFPR/DMV),VIVIEN MIDORI MORIKAWA(UFPR - DSC). Dando início à sessão, a presidência passou a palavra a(o) discente, para que a mesma expusesse seu trabalho aos presentes. Em seguida, a presidência passou a palavra a cada um dos Examinadores, para suas respectivas arguições. A aluna respondeu a cada um dos arguidores. A presidência retomou a palavra para suas considerações finais. A Banca Examinadora, então, e, após a discussão de suas avaliações, decidiu-se pela APROVAÇÃO da aluna. A Mestranda foi convidada a ingressar novamente na sala, bem como os demais assistentes, após o que a presidência fez a leitura do Parecer da Banca Examinadora. A aprovação no rito de defesa deverá ser homologada pelo Colegiado do programa, mediante o atendimento de todas as indicações e correções solicitadas pela banca dentro dos prazos regimentais do programa. A outorga do título de Mestre está condicionada ao atendimento de todos os requisitos e prazos determinados no regimento do Programa de Pós-Graduação. Nada mais havendo a tratar a presidência deu por encerrada a sessão, da qual eu, ALEXANDER WELKER BIONDO, lavrei a presente ata, que vai assinada por mim e pelos membros da Comissão Examinadora.

Observações: TÍTULO CORRIGIDO: SEROSURVEY OF BRUCELOSIS IN COMMERCIAL GOATS AND CART HORSES

Curitiba, 01 de Fevereiro de 2018.

ALEXANDER WELKER BIONDO(UFPR)
(Presidente da Banca Examinadora)

JOAO HENRIQUE PEROTTA(UFPR/DMV)

VIVIEN MIDORI MORIKAWA(UFPR - DSC)

AGRADECIMENTOS

Aos meus pais, Sandoval e Célia, devo agradecimentos eternos. Não só essa, mas todas as minhas conquistas só foram possíveis por conta do apoio incondicional que sempre tive.

Agradeço ao meu filho Martim que talvez tenha ficado um tanto de lado nesse período, mas é quem me dá força e inspiração.

Ao amigo e orientador, Dr. Alexander W. Biondo, por me receber tão prontamente como sua orientada, acreditando em mim e no meu trabalho.

A amiga Mara Lucia Gravinatti sou grata por tudo que fez por mim nesse processo, mesmo sem perceber. Não sei se conseguiria sozinha.

Ao Tecpar, instituição onde exerço minha profissão de Médica Veterinária tentando nunca perder o foco, e seus diretores, agradeço pelo apoio nessa jornada.

A amiga Giselle Nocera, por literalmente assinar embaixo que seria possível continuar o trabalho a seu lado e retomar os estudos, obrigada.

Agradeço ao meu coorientador Rafael Vieira e sua esposa Thállitha pelo auxílio sempre que preciso.

Ao querido professor Ivan Barros e ao professor João Perotta pela ajuda no atendimento aos equinos de Foz do Iguaçu. Ao Médico Veterinário André Souza Leandro por nos acolher no Centro de Controle de Zoonoses.

Aos alunos de iniciação científica da UFPR, residentes, mestrandos e doutarandos desse grupo e aos colegas de Tecpar, sempre dispostos a trabalhar em conjunto, meu “muito obrigada”.

A Deus e aos anjos que me protegem sou grata por tudo que já conquistei, por aquilo que está por vir e por colocarem em meu caminho pessoas e animais tão incríveis.

RESUMO

A brucelose bovina tem sido considerada endêmica em países em desenvolvimento causando problemas reprodutivos nesses animais e portanto gerando grandes prejuízos econômicos, principalmente nos países onde o agronegócio se mostra economicamente importante. As autoridades sanitárias têm desenvolvido programas de controle e erradicação da doença a exemplo dos países desenvolvidos onde a doença já está controlada ou erradicada. No entanto, mesmo com todos os esforços a brucelose humana vem sendo apontada como emergente em países em desenvolvimento. O presente trabalho objetiva demonstrar estudos sorológicos de diferentes espécies de *brucellas* em diferentes espécies animais as quais não têm sido contempladas nos programas sanitários destes países. Nesse sentido, o trabalho foi dividido em três capítulos. O Primeiro, um texto de opinião demonstrando como as autoridades, em diversas esferas do governo, vêm tratando a brucelose no Brasil. O segundo, um estudo sorológico de anticospos anti- B*rucella abortus* e anti-B*rucella ovis* em caprinos de diversas propriedades em municípios da Paraíba, onde foi encontrada a prevalência de 4,83%. E finalmente o terceiro, estudo sorológico de anticorpos anti-B*rucella abortus* em cavalos de carroça em Foz do Iguaçu, Paraná encontrando 1,64% de prevalência.

Palavras chave: saúde única; equinos; caprinos; zoonose; cavalo de carroça; brucelose; vulnerável

ABSTRACT

Bovine brucellosis has been considered endemic in developing countries causing reproductive problems in these animals and thus generating major economic losses, especially in countries where agribusiness is economically important. The health authorities have developed programmes for the control and eradication of the disease in the case of developed countries where the disease is already controlled or eradicated. However, even with all the efforts human brucellosis has been pointed out as emerging in developing countries. The present work aims to demonstrate serological studies of different species of *brucellas* in different animal species which have not been contemplated in the sanitary programmes of these countries. In this sense, the work was divided into three chapters. The first, a text of opinion demonstrating how the authorities in various spheres of the government have been treating brucellosis in Brazil. The second, a serological study of Anticospos anti-Brucella abortus and Abortus Ovis in goats of several properties in municipalities of Paraíba, where the prevalence of 4.83% was found. And finally the third, serological study of antibodies *Brucella abortus* in wagon horses in Foz do Iguaçu, Paraná meeting 1.64% of prevalence.

Keywords: one health; equines; goats; zoonosis; cart horse; brucellosis; vulnerable

LIST OF FIGURES

FIGURA 1 ZOONOSES CITED BY THE MINISTRIES OF HEALTH AND AGRICULTURE

FIGURA 2 TRANSMISSION MODE

FIGURA 3 NORTHEASTERN COMMERCIAL GOATS, BRAZIL

FIGURA 4 SITUATION OF CART HORSES IN FOZ DO IGUAÇU, PR

FIGURA 5 SITUATION OF CART HORSES IN FOZ DO IGUAÇU, PR

LIST OF TABLES

TABLE 1 RISK CLASSIFICATION TABLE FOR BOVINE AND BUFFALO BRUCELLOSIS

TABLE 2 SEROPREVALENCE OF *BRUCELLA OVIS* ON EIGHT DIFFERENT GOAT FARMS IN THE STATE OF PARAÍBA, NORTHEASTERN BRAZIL, AND THE CORRESPONDING TESTING OF RISK FACTORS

TABLE 3 SEROPREVALENCE OF *BRUCELLA ABORTUS* IN CART HORSES FROM FOZ DO IGUAÇU, SOUTHERN BRAZIL AND CORRESPONDENT TESTING OF RISK FACTORS

TABLE 4 SEROPREVALENCE OF *BRUCELLA ABORTUS* IN CART HORSES FROM FOZ DO IGUAÇU, SOUTHERN BRAZIL AND CORRESPONDENT TESTING OF RISK FACTORS

LIST OF ABBREVIATIONS

AGID	Agarose Gel Immunodiffusion
CI	Confidence Interval
DOU	Federal Official Gazette
EMBRAPA	Brazilian Agricultural Research Corporation
GTA	Animal Traffic Guide
IBAMA	Brazilian Institute of Environment and Natural Resources
IBGE	Brazilian Institute of Geography and Statistics
IN	Normative Instruction
MAPA	Ministry of Agriculture Livestock and Food Supply
2-ME	2-Mercaptoetanol Test
MS	Ministry Of Health
OR	Odds Ratio
PAHO	Pan American Health Organization
PANAFTOSA	Pan American Foot-and-Mouth Disease Center
PNCEBT	National Program For The Control And Eradication Of Brucellosis And Tuberculosis
RBT	Rose Bengal Test
SBMT	Brazilian Society Of Tropical Medicine
SEDAP-PB	State Secretary for Agricultural Development and Fisheries-Paraíba
SESA-PR	State Secretary of Health-Paraná
UF	Federative Units
UFPR	Federal University of Parana

SUMARY

1. INTRODUCTION 13
REFERENCES 14
1.2 OBJECTIVES 15
1.3 HYPOTHESIS 16
2. BRUCELOSIS IN BRAZIL 16
2.1 ENDEMIC OR EMERGING DISEASE 16
2.2 HEALTH PROGRAMS – PNCEBT 17
2.2.1 WHAT ACTUALLY CHANGES IN THE NEW REVISION 18
2.2.2 IN ADDITION TO THIS CLASSIFICATION, OTHER CHANGES ARE WORTH MENTIONING (MAPA, 2017) 19
2.3 EPIDEMIOLOGICAL DIAGNOSIS AND CONCLUSIONS 20
REFERENCES 22
3. SEROEPIDEMIOLOGY OF *BRUCELLA OVIS* AND *BRUCELLA ABORTUS* IN GOATS THE STATE OF PARAÍBA, NORTHEASTERN BRAZIL 24
3.1 ABSTRACT 24
3.2 INTRODUCTION 24
3.3 MATERIALS AND METHODS 25
3.4 RESULTS 27
3.5 DISCUSSION 29
3.6 CONCLUSION 30
REFERENCES 31
4. SEROSURVEY OF *BRUCELLA ABORTUS* ANTIBODIES IN CARTHORSES FROM FOZ DO IGUAÇU, IN THE TRI-BORDER REGION OF BRAZIL 34
4.1 ABSTRACT 34
4.2 INTRODUCTION 34
4.3 MATERIALS AND METHODS 36
4.4 RESULTS 37
4.5 DISCUSSION 39
4.6 CONCLUSION 40
REFERENCES 40
5. GENERAL CONCLUSIONS 42
6. REFERENCES 42
7. SUPPLEMENTS 49
8. VITA 53

1. INTRODUCTION

Brucellosis has been characterized by a zoonotic, infecto-contagious disease caused by bacteria of the genus *Brucella spp*, and may post threating impact on animal and public health. Over 500,000 cases of brucellosis have been yearly reported in human beings throughout the world (MOLYNEUX et al, 2011).

The disease distribution has been related to the concentration of cattle and their contamination, which even with efforts for brucellosis control, still remains an economic and sanitary issue for cattle herds from several countries (MUFINDA et al, 2017).

Brucellosis has been considered to be neglected and endemic in the middle east countries, saudi arabia and the mediterranean, where infected ruminants have been the main source of human infection and, consequently, estimatives of brucellosis frequency in ruminants may be useful for applying effective control strategies (HEGAZY et al., 2011 and ELIZEI et al, 2010). Although higher notification rates have been reported in mediterranean countries, infection has been considered rare in human beings of the European Union (GAROFOLO et al., 2016; GAMBOA et al, 2016).

Brucellosis has been reported in latin america since the first decade of the 20th century and remains even today as an important zoonosis, despite the campaigns and other efforts for control and prevention. Persistence and distribution of disease in different animal species has been facilitated by the geographical, climatic and economic conditions of the area (LUCERO et al, 2008).

Brucellas are reportedly intracellular, gram-negative bacteria, which can infect several animal species, mostly caused by *Brucella abortus*, *Brucella melitensis*, *Brucella suis*, *Brucella ovis*, *Brucella canis* and *Brucella neotomae*. Such classification has been mainly based on the differences in pathogenicity and host preference. The main worldwide pathogenic species has been *B. Abortus*, responsible for bovine brucellosis; *B. Melitensis*, the main agent etiological of ovine and caprine brucellosis; and *B. suis*, responsible for swine brucellosis (GODFROID et al, 2010).

Although Bovines have reportedly been the preferred hosts of *Brucella abortus*, other animals, including bison, camels, as well as equideos, swine, goats, buffaloes and dogs have also been commonly infected (ELISEI et al., 2010).

The presence of *Brucella* among wild animals has turned wildlife as a source of infection for domestic animals and human beings, thus prevalence of infection in animal

reservoirs may directly correpond to occurrence in contacting people (ACHA and SYFRES, 2003; ELISEI et al. 2010).

Bovine brucellosis eradication programmes in the european union and the united states have emphasized the identification of potential wild reservoirs for *Brucella abortus*. Countries where eradication programmes have succeeded or considered in advanced stages, such reservoirs have been identified and controlled (GODFROID et al, 2010).

Despite continuous advances on disease control and prevention, habits of consumption of raw or non-heat-treated foods, handling of animals, animal organs, animal products and excretions without precautions and protective equipment has kept brucellosis as an important public health threat throughout the world, particularly in countries lacking basic hygiene on livestock (FRANC et al, 2018).

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1.2 OBJECTIVES

GENERAL OBJECTIVE

Determine the seroprevalence of *Brucella spp* antibodies in differents animals.

SPECIFIC OBJECTIVES

Determine the soroprevalence of *Brucella spp* antibodies in goats of the Paraíba, region that accounts the national goat population and largest producer of goat's milk.

Determine the soroprevlence of *Brucella spp* antibiodes in cart horses from Foz do Iguaçu, in the tri-border region of Brazil.

1.3 HYPOTHESIS

Goats and cart horses are potential reservoirs of *Brucella spp* and may be epidemiologically important in the transmission of human brucellosis.

2. BRUCELLOSIS IN BRAZIL

Subject

2.1 ENDEMIC OR EMERGING DISEASE

Causing significant economic and health damage, especially in herds, brucellosis is a neglected zoonosis caused by bacteria of the genus *Brucella spp*., which also affects pets, and according to the World Health Organization (WHO) under-reported in human (LAWINSKI et al, 2010).

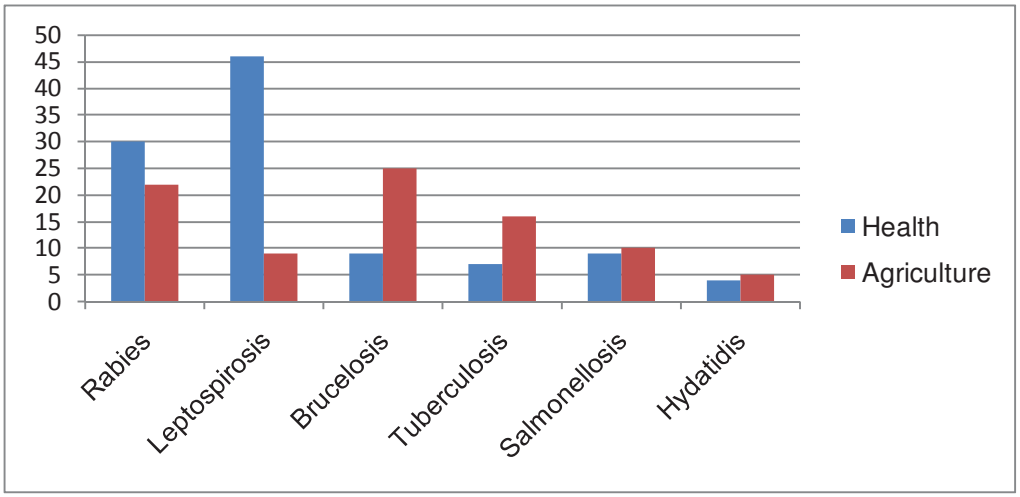
The infectious physician, Marcos Vinicius da Silva, a human brucellosis researcher, points out his concern about the increasing number of cases, due to the lack of knowledge of the disease by health professionals and the non-specific symptomatology, because of it is necessary good information around epidemiological antecedents for a required suspicion (SBMT, 2017), culminating in a failure of the notification system.

The positivity of cases of brucellosis in humans occurs in three distinct risk populations: accidents involving professionals in laboratories, professionals in cattle management and consumers of unpasteurized dairy products (LAWINSKI et al, 2010; MAXWEL et al, 2017).

In 2017, the Pan American Health Organization (PAHO), through the Pan American Foot-and-Mouth Disease Center (PANAFTOSA), concluded the survey of emerging and endemic zoonoses of high importance to the Ministries of Health and Agriculture in 33 Latin American and Caribbean countries, indicating that the impact is greater in neglected populations. Of these, 31 countries responded to the questions, including Argentina, Bahamas, Barbados, Bermuda, Brazil, Bolivia, Cayman Islands, Chile, Colombia, Cuba, Dominican Republic, Ecuador, El Salvador, Mexico, Nicaragua, Panama, Paraguay, Peru, Saint Lucia, Saint Vincent and the Grenadines, Suriname, Uruguay, Trinidad and Tobago, Turks and Caicos Islands and Venezuela (LAWINSKI et al, 2010; MAXWEL et al, 2017).

Research has shown that brucellosis is considered one of the three emerging zoonoses most cited by the Ministries of Health surveyed and the main endemic zoonosis cited by the Ministries of Agriculture (Figure 1), converging to the need for the integration and alignment of the two Ministries in carrying out epidemiological surveys of important zoonosis (MAXWEL et al, 2017).

Figure 1 – Zoonoses cited by the Ministries of Health and Agriculture



Source: MAXWEL et al, 2010

2.2 HEALTH PROGRAMS – PNCEBT

In June 2017, the "Technical Regulation of the National Program for the Control and Eradication of Brucellosis and Tuberculosis (PNCEBT)" was published in Federal Official Gazette (DOU) by Normative Instruction 10/2017 of the National Secretariat of Agricultural and Livestock Ministry of Agriculture, Livestock and Supply (MAPA). The Program had recently been updated through Normative Instruction 19, repealed at the end of 2016, and provides guidelines in an attempt to reduce and eradicate the disease.

The validity of this new program was established after a technical cooperation agreement between MAPA and the Faculty of Veterinary Medicine and Animal Science of the University of São Paulo, published in the Federal Official Gazette on January 1, 2003, case 21000.009003 / 2002-59. This objective includes epidemiological studies within the PNCEBT, such as the characterization of the epidemiological situation of brucellosis and bovine tuberculosis in the brazilian Federal Units (UF).

In these studies, factors and variables that could be associated with the prevalence of the disease in the federative units were considered. Among the analyzed variables, we can highlight: number of cattle, system of exploitation (meat, milk and mixed), type of breeding (confined, semi-confined, extensive), use of artificial insemination, predominant breeds, presence of other domestic species, presence of wild animals, destination of placenta and aborted fetuses, purchase and sale of animals, vaccinations against brucellosis, slaughter of animals on the property, pasture rental, common pastures with other properties, flooded pastures, picket fodder and veterinary assistance (POESTER et al, 2009).

2.2.1 WHAT ACTUALLY CHANGES IN THE NEW REVISION

Table 1 represents the strategy of PNCEBT performance based on the classification of the federative units (UF) as to the degree of risk for diseases, and on the definition of animal health protection procedures to be adopted according to this classification, where E0 - Unknown Risk; D0, D1, D2 and D3 - High risk; C0, C1, C2 and C3 - Average risk; B0, B1, B2 - Low risk; B3, A0, A1 and A2 - Very low risk; A3 - Low risk.

Table 1 - Risk classification table for bovine and buffalo brucellosis

Prevalence Outbreaks (%)	**Class**	**Level**			
		Initial	**Qualification of the execution of the actions**		
			Low	**Average**	**High**
<2	THE	0	1	2	3
≥ 2 <5	B	0	1	2	3
≥ 5 <10	W	0	1	2	3
≥ 10	D	0	1	2	3
Unknown	AND	0	0	0	0

Source: Normative Instruction 10 of June 20, 2017 / MAPA

- E0 - Unknown risk: vaccination against brucellosis with vaccine coverage of animals over 80% and epidemiological study of brucellosis;

- D0, D1, D2 and D3 - High risk: vaccination against brucellosis with vaccination coverage of animals above 80%;

- C0, C1, C2 and C3 - Average risk: vaccination against brucellosis with vaccination coverage of animals over 80%;

- B0, B1, B2 - Low risk: vaccination against brucellosis with vaccination coverage of animals above 80%;compulsory clearing of detected outbreaks; and epidemiological surveillance for foci detection;

- B3, A0, A1 and A2 - Very low risk: mandatory sanitation of the detected outbreaks and epidemiological surveillance for foci detection.

- A3 - negligible risk: mandatory sanitation of detected outbreaks and epidemiological surveillance for foci detection.

A tabela 1 representa a estratégia de atuação do PNCEBT baseada na classificação das unidades federativas (UF) quanto a prevalência em foco (um ou mais animais positivos por propriedade) e as ações executadas no combate a doença. Ou seja, a definição de procedimentos de defesa sanitária animal a serem adotados será: os estados que receberem a classificação E (prevalência desconhecida) deverão realizar estudos epidemiológicos para conhecerem sua prevalência além de ser obrigatória a cobertura vacinal de 80% do rebanho; os classificados em D e C (prevalências conhecidas acima de 10% e entre 5 a 10% respectivamente) deverão realizar cobertura vacinal de 80% do rebanho até que a prevalência diminua; os classificados em B (prevalência entre 2 a 5%) deverão realizar cobertura vacinal de 80% do rebanho, saneamento obrigatório de focos detectáveis e vigilância epidemiológica para detecção de focos; e os classificados em A (prevalência abaixo de 2%) poderão ser livres de vacinação mas com saneamento obrigatório de focos detectáveis e vigilância epidemiológica para detecção de focos.

2.2.2 IN ADDITION TO THIS CLASSIFICATION, OTHER CHANGES ARE WORTH MENTIONING (MAPA, 2017)

1.The Federal Units will be entrusted with the creation of*"State Commissions to Combat Brucellosis and Tuberculosis"*, which should assists the creation of state public

policies for the feasibility of disease control procedures and in the elaboration and maintenance of funds for indemnity of the rural producer whose animals are slaughtered due to the diagnosis.

2. The issuance of an Animal Transit Guide (GTA) for interstate displacement of cattle and buffaloes, whatever the purpose and not only for reproduction, shall be conditioned to negative diagnostic tests depending on the Federative Unit that the animal is destined for. Only those States classified as very low or negligible risk will continue to submit the tests only for animals intended for breeding.

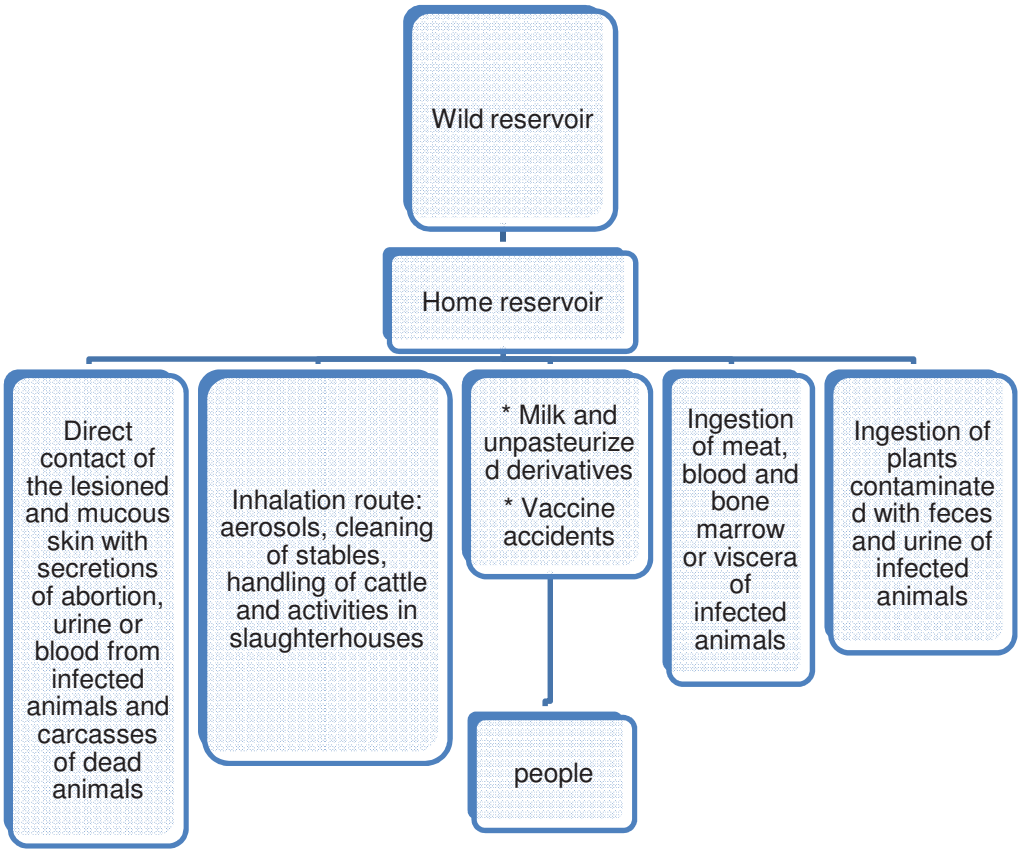
3. Vaccination and labeling in the animals also underwent changes, the main one being the non-antibody-inducing vaccine, Rb51, even in young females. Being the most critical point of the changes, because as already said the vaccine accidents are still cause of human brucellosis.

2.3 EPIDEMIOLOGICAL DIAGNOSIS AND CONCLUSIONS

Shortly after the revision and publication of the new PNCEBT, the event "Brucellosis: Diagnoses and Zoonoses" was held in Castro, an important dairy basin of Paraná on a demand from the Sanitary Surveillance of the municipality due to the increased notification of cases of brucellosis in the region, which is the largest dairy basin in the state of Paraná.

It is worth remembering that the notifications gained attention after the establishment of the "Protocol of Clinical Management and Health Surveillance for Human Brucellosis in the State of Paraná" (SESA-PR, 2016)). States such as Santa Catarina and Tocantins have similar or finalizing protocols, respectively, demonstrating the importance of the flowchart (figure 2) and real knowledge of the disease dimension, as well as guiding the actions of health professionals to determine the source of streaming.

Figure 2 - Transmission mode



Source: SESA-PR, 2016

One of the major concerns of the Health Secretaries is the use of the Rb51 vaccine by professionals in the risk areas, since it is formulated with an attenuated sample of Brucella abortus rugosa, originated from a virulent, mutated and successive sample passages. Because it is a rough sample, it does not induce the formation of anti-LPS smooth antibodies, which makes routine serological tests for Brucella sp. , based on the detection of these antibodies, are not successful (LAWINSKI et al, 2010). As a result, the protocol does not include species that are important for public health, such as Brucella canis.

The occupations and exposure considered at risk, according to the Paraná protocol, are:

a) Professionals for the management of live and slaughtered animals: animal handlers, veterinarians and their auxiliaries, agriculturalists, among others;

(b) slaughterhouse / slaughterhouse workers during slaughtering and handling of meat and offal products;

(c) milking and dairy workers and similar activities;

d) Accidents during the application of vaccines: by dermal inoculation during the application of the animal vaccine or contact of the vaccine liquid in the mucous membranes;

e) Laboratory accident: manipulation of cultures of bacteria, aspiration of cultures and aerosols, direct contact with the skin and conjunctiva;

f) Manipulation of biological material by health professionals.

However, the protocol does not include students of Veterinary Medicine, although several reports have reported contamination in this population (PEPPER, 2017) causing them to ask the following question: why not require certification on all farms of universities? It does not include controllers of invasive species, an activity regulated by IBAMA (IBAMA, 2013)) as risk groups. Despite of this case there are efforts of EMBRAPA researchers to offer training to these people so they can protect themselves.

All these considerations lead us to think how far we are from controlling this disease. Brazil needs a greater integration between the Ministry of Agriculture and Food Supply (MAPA) and the Ministry of Health (MS), as well as other Body such as IBAMA and EMBRAPA, so that the information does not get out of hand and that control strategies diseases such as brucellosis are well established.

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3. SEROEPIDEMIOLOGY OF *BRUCELLA OVIS* AND *BRUCELLA ABORTUS* IN GOATS IN THE STATE OF PARAÍBA, NORTHEASTERN BRAZIL

Subject

3.1 ABSTRACT

The aim of this study was to identify the seroprevalence of *Brucella ovis* and *Brucella abortus* antibodies and potential factors associated with infection in goats in the state of Paraíba, northeastern Brazil, the region that accounts for 90.98% of the national goat population. Samples were collected between May and November 2014, stored at -80°C and sent for analysis. The seroprevalence of anti-*Brucella ovis* antibodies was measured using the agar gel immunodiffusion test (AGID); and anti-*Brucella abortus* antibodies using the Rose Bengal test (RBT), with confirmation using the 2-mercaptoethanol (2-ME) test. Overall 18/373 samples (4.83%) were positive for anti-*Brucella ovis*. Although 5/373 samples (1.34%) were weakly positive according to RBT, there was no confirmation from 2-ME and therefore all samples were considered negative for anti-*Brucella abortus*. In the region with the biggest Brazilian herd may pose a threat to animal and public health and should be further investigated and monitored. In addition, 2-ME should always be performed as a confirmation test for *Brucella abortus* detection.

3.2 INTRODUCTION

Brucellosis is one of the most common zoonotic bacterial diseases affecting domestic and wild animals worldwide. It is caused by at least a dozen *Brucella* species including *B. melitensis* (sheep and goats, humans), *B. abortus* (cattle, humans) and *B. suis* (pigs), which are also serious infectious agents for human; *B. canis* (dogs), which is mildly infectious for humans; and *B. ovis* (sheep), which is non-infectious for humans (GWIDA et al, 2010). Because livestock form important reservoirs for human infections, this has been the focus for endemic control and disease eradication in several countries (SADHU et al, 2015).

It has been reported that brucellosis is spreading among small ruminants. This has probably been due to growth in trading and rapid animal movements, together with overlapping between areas with sheep and goat herds (SADHU et al, 2015). Although goat

infection by *B. melitensis* has never been reported in Brazil, *B. abortus* has been sporadically described and may represent a risk of human infection (LILENBAUM et al, 2007). On the other hand, ovine brucellosis has been considered endemic in Brazil, given that 3/50 sheep flocks (6.0%) in southeastern Brazil were found to be positive (COSTA et al, 2016) and 52/1,800 rams (2.89%) in southern Brazil were found to be positive (MACHADO et al, 2015). Ovine brucellosis may also affect goats, even though it is not considered zoonotic (OIE, 2015).

Northeastern Brazil has been ranked first in the goat head count among the country's regions, accounting for 90.89% of the national goat population (SEDAP-PB, 2017). The state of Paraiba stands out within this region as having the largest commercial dairy goat production. This is mostly focused on family subsistence and is responsible for approximately 5 million liters of milk per year (SEDAP-PB, 2017). A goat milk program was established in 2003 to benefit local low-income dairy goat farmers, with the aim of guaranteeing a market and fair product prices (SEDAP-PB, 2003).

Brucellosis may be transmitted through direct contact with herds or consumption of contaminated products (PINHEIRO-JUNIOR et al, 2008), and thus it presents potential risks to both animal and public health. However, no epidemiological studies have been conducted to date on the seroprevalence of *Brucella spp*. among goats in northeastern Brazil. Accordingly, the present study had the aim of serologically surveying anti-*B. ovis* and anti-*B. abortus* antibodies and associated factors on goat farms in the state of Paraiba, northeastern Brazil.

3.3 MATERIALS AND METHODS

This study was approved by the Ethics Committee for Animal Experimentation and Animal Welfare at the Federal University of Paraíba (Protocol 3305/14), and was conducted in accordance with the ethical principles of the Brazilian College of Animal Experimentation. A total of 373 serum samples (341 from female goats and 32 from male goats) from eight goat farms that had previously been surveyed for other pathogens (BRAZ et al, 2017) were included in the present study. All samples were stored at -80 °C until serological procedures were performed.

An epidemiological questionnaire was applied to all the farm owners, which included questions on sex and age, abortion, mummified fetuses and the production system of each goat farm. The goats were age-stratified into groups of ≤ and > 1 year. A goat meat farm

at Juarez Távora was taken to be an example of high-quality management practices and was used as a reference farm for statistical analysis.

Diagnostic methods were applied in accordance with the official recommendations for controlling ovine epididymitis and bovine brucellosis (MAPA, NI, 2016), respectively. Detection of antibodies against *B. ovis* was performed using a commercially available kit (agar gel immunodiffusion test, AGID; Tecpar, Curitiba, Brazil), in accordance with the manufacturers recommendations, using lipopolysaccharides and protein antigens of *B. ovis*, strain Reo 198 (antigen for *Brucella ovis* diagnosis, Tecpar, Curitiba, Brazil). Detection of *B. abortus* antibodies was performed using the Rose Bengal test (RBT) for screening and the 2mercaptoethanol test (2-ME) for confirmation, with an inactivated cell suspension of *B. abortus*, strain 1119-3 (RBT for brucellosis diagnosis, Tecpar, Curitiba, Brazil). As mentioned above, all tests were conducted following national and international protocols and recommendations (OIE, 2015; MAPA, 2017).

The chi-square or Fisher's exact test was used to determine potential associations between individual factors and seropositivity for Brucella spp. The odds ratio (OR), 95% confidence interval (CI) and p-value were calculated for each variable. Results were considered to be significantly different when $p < 0.05$. The data were compiled and analyzed using freely available software (Epi Info, version 7.1.5, CDC, Atlanta, GA, USA).

Figure 3 - Northeastern Commercial Goats, Brazil

Source: Rafael F.C. Vieira, 2014

3.4 RESULTS

The seroprevalence of *B. ovis* in goats and associated factors are presented in Table 1. Out of the total of 373 goats, 18 (4.83%; 95% CI: 3.07-7.50%) were seropositive for *B. ovis*, and eight farms 6/8 (75%) surveyed presented at least one seropositive animal. No significant association was found between seropositivity for *B. ovis* and age, sex, abortion, mummified fetuses, production system or multispecies grazing ($p > 0.05$). A total of 5/373 goat serum samples (1.34%; 95% CI: 0.573.10%) tested weakly positive for *B. abortus* by means of RBT, but none of these samples was confirmed as positive through 2-ME. Thus, all the goats were considered to be seronegative for *B. Abortus.*

Table 2 - Seroprevalence of *Brucella ovis* on eight different goat farms in the state of Paraíba, northeastern Brazil, and the corresponding testing of risk factors

Farm	**Production system**	**+/n**	**(%)**	**OR**	**95% CI**	**P-value**
Algodão	Dairy	0/29	0.00	0.0000	-	0.53719
Caturité I	Dairy	1/45	2.22	0.5795	0.05-6.61	1.00000
Caturité II	Dairy	4/56	7.14	1.9615	0.34-11.18	0.67932
Gurjão	Dairy	0/36	0.00	0.0000	-	0.51277
Serra Branca	Dairy	4/60	6.67	1.8214	0.32-10.37	0.68271
Cuité	Goat meat	3/51	5.88	1.5938	0.25-9.96	0.67521
Olivedos	Goat meat	4/43	9.30	2.6154	0.45-15.02	0.40292
Juarez*	Goat meat*	2/53	3.77			
Age	> 1	16/310	5.16	1.6599	0.37-7.41	0.74883
	≤ 1	2/63	3.17			
Gender	Male	1/32	3.13	0.6148	0.08-4.78	1.00000
	Female	17/341	4.99			
Abortion	Yes	17/328	5.18	2.4051	0.31-18.52	0.70885
	No	1/45	2.22			
Mummified fetuses	Yes	4/56	7.14	1.6648	0.53-5.25	0.32728
	No	14/317	4.42			
Production system	Dairy	9/225	4.00	0.6435	0.25-1.66	0.35887
	Goat meat	9/148	6.08			
Multispecies grazing	Yes	14/277	5.05	1.2243	0.39-3.81	1.00000
	No	4/96	4.17			

+, Number of positive animals; n, number of samples; 95% CI, 95% confidence interval. * Reference goat farm

3.5 DISCUSSION

To the best of our knowledge, this study may be the first report of *Brucella spp*. in goats in Paraíba. There are 9.000.000 goats in Brazil, the northeast being the largest producer and Paraíba is the largest producer of goat milk (IBGE, 2016).

Data on sheep have been more frequently reported in Brazil, and some factors associated with *Brucella* infection have been identified (MENDONÇA et al, 2017). The proportion of the goats found here to be positive for *B. ovis* infection (18/373; 4,83%) was to the proportions of sheep previously found to be positive in Paraíba (6/80; 7.5%) and Sergipe (41/932; 4.40%) but lower than in Piauí 16/90; 17,8%), all in northeastern Brazil (MENDONÇA et al, 2017; ALVES et al, 2010; COSTA et al, 2012) .

In other countries, the seroprevalence in sample obtained from all regions of Croatia was 202/22.686 (0,9%) (SPICIC et al, 2010) while in sample obtained in Quebec, Canada, was entirely seronegative for *B. ovis* (ARSENAULT et al, 2004).

Previous studies have suggested that associations of direct diagnostic methods, culturing and clinical evaluation may necessary for achieving efficient diagnosis of *B. ovis* infection. Serological tests (AGID) have been indicated as the most feasible method for diagnosing *B. ovis*, because of their better degree of simplicity cost-benefit and sensitivity (OIE, 2015). Interestingly, all the female goats that were seropositive for *B. ovis* in the AGID test in the present study had had histories of reproductive problems such as weak live births, stillbirths or abortions.

The prevalence of *Brucella sp* in goat herds in the present study may have been a consequence of the farms low technical and sanitary levels. These farms were located in low-income areas of the goat milk program that had been set up to benefit local dairy goat farmers (SEDAP-PB, 2003). This scenario of poor small-ruminant farm management may have favored infectious and parasitic diseases, through lack of the hygiene procedures that comprise the basic recommended measures for supporting effective disease control and prevention (COSTA et al, 2012).

All 373 samples were seronegative for *B. abortus*. Similar negative results had previously been reported in Bahia, while the seropositive rate among goats in the state of Pernambuco was only 2/340 (0.6%). This result from Pernambuco state may have been biased, since only a modified Rose Bengal test (RBT) with a serum-toantigen ratio of 3:1 was used, with no confirmatory tests. Likewise, in Rio de Janeiro, southeastern Brazil, 5/953 (0.5%) of the samples were found to be positive, but only anti-*B. abortus* agglutinins

were used (RBT), without confirmatory tests (LILENBAUM et al, 2007; PINHEIRO-JUNIOR et al, 2008; PEIXOTO et al, 2016).

Although the statistical analysis did not show any correlation between grazing species and seropositivity for *Brucella spp* ($p > 0.05$), 14/277 goats were raised in this system and presented positive results for *B. ovis*. In addition, although the main activity of the farms in the present study consisted of sheep and goat-grazing and no sample was positive for *B. abortus*, it has previously been reported that confirmatory tests are inconclusive (PINHEIRO-JUNIOR et al, 2008). Furthermore, the dilutions used in the 2-ME test were performed in accordance with the recommendations for cattle in the national program for control and eradication of animal brucellosis and tuberculosis (MAPA, NI, 2016). Further studies need to be conducted to fully establish the appropriate dilutions for samples from goats.

The epidemiological results from the goat serological survey of the present study may indicate that there is a lack of sanitary programs and monitoring strategies for controlling *Brucella* infections. Prevalence of goats with brucellosis may represent a high risk to small ruminants and to the corresponding farming families. Thus, there is a clear requirement for further studies on the impact of brucellosis on public health (RUSSO et al, 2016). The limitations of the present study may include a further differential diagnosis among positive goats, despite reproductive problems, given that other bacteria of rough membranes, such as *B. canis*, may cause cross-reactivity in the AGID test. In addition, although *B. abortus* antibodies were not identified in the confirmatory 2-ME test, the presence of weakly positive serum seen through the RBT (seroprevalence of 1.47%) may indicate that there is a need for standardized titration of goat samples, considering that the protocol applied was developed for cattle.

According to the Brazilian Ministry of Agriculture, no cases of brucellosis caused by *B. melitensis* have been reported to date in Brazil. However, serological studies and attempts to isolate the agent should continue to be conducted, given that there is a possibility that cross-reactions may have occurred in RBT tests (PINHEIRO-JUNIOR et al, 2008).

3.6 CONCLUSION

Prevalence of brucellosis in goats may represent a high risk to small ruminants, farming families and the respective goat milk market, particularly in regions with high

consumption. Thus, there is a clear need for further studies on the impact of brucellosis on public health. Although studies on the seroprevalence of *B. abortus* have often relied solely on the Rose Bengal test (RBT), the results from this test should be always confirmed by means of the 2-mercaptoethanol test (2-ME).

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4. SEROSURVEY OF *BRUCELLA ABORTUS* ANTIBODIES IN CARTHORSES FROM FOZ DO IGUAÇU, IN THE TRI-BORDER REGION OF BRAZIL.

Subject

4.1 ABSTRACT

Mostly studies on brucellosis have been conducted in cattle, sheep and goats, with few reports in domesticated non-ruminant species. Accordindly, the aim of present study was to establish the seroprevalence of *Brucella abortus* antibodies in cart horses from Foz do Iguaçú, tri-border region in Brazil. Blood samples were drawn and a epidemiological questionnaire was applied to owners for identifying potential risk factors. A total of 8/61 (13.11%) positive samples were found in the RBT test, of which 1/61 (1.64%) was confirmed as positive through 2-ME. Although the results found herein may not be sufficient to determine the epidemiological importance of horses as brucelosis reservoirs, further research should be conducted in these areas along owner serology. Horses may be used as sentinel animals of disease in urban areas, as a toll for outbreak prevention. The close contact of horses and their owners living in vulnerable conditions and lacking basic sanitation may post a risk to public health.

4.2 INTRODUCTION

Although many studies on brucellosis have been conducted in cattle, sheep and goats, particularly in cattle, few reports have been conducted to determine the epidemiological role of domesticated non-ruminant animals (GONZÁLES et al, 2006).

In most traditional breeding systems, urban and peri-urban areas, brucellosis probably doesn't have endemic stability. Because of this, it is necessary monitoring and estimating the prevalence of antibodies against the bacteria. In some African countries, in the Middle East and in Asia, livestock farming in urban and peri-urban environments are often characterized by low biosecurity where different animal species are kept in close proximity to human (GODFROID, 2017).

Brucellosis has been reported in Latin America since the first decade of the 20th century and remains to this day a major zoonosis despite the programs to control the disease. Its persistence and wide distribution in different animal hosts is facilitated by the

peculiar geographic, climatic and economic conditions of the area (LUCERO, SCOBAR 2008) .

Brucellosis is a zoonotic disease affecting humans and animals. The close contact between infected animals and susceptible hosts is a potential method for pathogen transmission. Cart horses in Brazil are a re-emerging group of animals in urban areas, with owners daily traffic across big cities and surroundings to collect and recycling for living, with low income and no access to veterinary assistance. This re-emerging population may present public health significance, and should be periodically monitored (ANTUNES et al, 2013).

Howewer, understanding the biology of *Brucella* infections and the transmission patterns at the wildlife/livestock/human interface is of paramount importance before implementing any brucellosis control or eradication program in animals, even more so should interventions be justified within One Health. In addition to calling for transdisciplinary collaboration, One Health formally aims to conserve the environment and to promote the well-being of animals and humans (GODFROID, 2017).

The cohabitation with other species is the most likely source of infection in horses, even in urban areas, where the animal can live with pigs, dogs, chickens, etc. Some species of *Brucella* already been identified in the equines, *Brucella suis* and the most common *Brucella abortus*. (ANTUNES, 2013).

Even the economic losses of *Brucella* spp infection in equines are considered lower than other animals of zootechnical interest. The illness has debilitating lesions, it is recommended a sacrifice of positive animals and infected animals represent an important source of infection to other species and humans (RADOSTITS et al, 2000).

Brucellosis in horses is characterized by three conditions poll evil, fistulous withers, and rarely late abortions in infected mares. Pathogen has its predilection for joints, ligaments and tendons in case of equines and causes inflammatory conditions leading to formation of fistula. This fistula should be taken into consideration as an environmental contamination factor, because the exudate content is highly rich in viable bacteria that can infected others species (FERNADES et al, 2014).

Different methods are used to diagnose brucellosis in horses, but for an unequivocal diagnosis, isolation of the pathogen from articular lesions or ligaments is required. Difficulty in isolating the etiological agent in lesions, because of contamination by opportunistic organisms, is considered to be a limiting factor in making the diagnosis of equine brucellosis (RIBEIRO et al, 2003).

In a study carried out in Latin America, 1377 strains of *Brucella spp* were isolated in humans and different animal species. *B. melitensis*, known as the most pathogenic species, was the one most frequently isolated from humans, followed by *B. suis* and B. a*bortus*. In Argentina, the species most isolated was *B. suis* (LUCERO et al, 2008)**.** In the tri-border region where Brazil meets Paraguay and Argentina, the constant flow of people and animals across national borders makes this region particularly vulnerable.

The aim of present study was therefore to establish the seroprevalence of *Brucella abortus* antibodies in carter horses in the tri-border region of Brazil.

4.3 MATERIALS AND METHODS

The survey was performed in municipality Foz do Iguaçu, Paraná State. Horse blood samples were collected from animals whose owners are volunteer assistance in project "Cavalos Carroceiros" (Universidade federal do Paraná - UFPR). After registering the address of each animal, the samples collections were made in the environment where these animals lived. A questionnaire for epidemiological patterns was administered for identifying probable risk factors. Subsequently, according to the National Program for the Control and Eradication of Brucellosis and Tuberculosis of the Ministry of Agriculture, Livestock and Supply (PNCEBT - MAPA), these samples were tested with Rose Bengal test (RBT) that permits the serological diagnosis of brucellosis by rapid agglutination. Sera from the positive and weak positive animals in RBT were submitted to the slow agglutination test by 2-mercaptoethanol for confirmation of infection in samples.

Figure 4 - Situation of Cart Horses in Foz do Iguaçu, PR

Source: Ivan Barros, 2016

Figure 5 - Situation of Cart Horses In Foz do Iguaçu, PR

Source: Ivan Barros, 2016

4.4 RESULTS

The results of the serology are displayed in Tables 1 and 2. A total of 8/61 (13.11%) positive samples were found in the RBT, of which 1/61 (1.64%) was serologically confirmed by slow serum agglutination.

The positive animal is a female, 8-year-old, infant, had a two years ago an abortion and living with other animals (dogs, birds and pigs), acccording epidemiological questionnaire. The others 8 positive animals by RBT test, two of them also coexist with other species.

Table 3 - Seroprevalence of *B. abortus* in cart horses from Foz do Iguaçu and correspondent testing of risk factors

***B. abortus* (Rose Bengal Test)**			
		+/n	**(%)**
Age	>5	7/39	17.95
	≤5	1/22	4.55
Gender	Male	1/30	3.33
	Female	7/31	22.58
Abortion	Yes	2/3	66.67
	No	5/28	17.86
Graze	Yes	8/60	13.33
	No	0/1	0.00
Multispecies	Yes	6/48	12.50
grazing	No	2/13	15.38

+/n, Number of positive animals; %, soroprevalence

Table 4 - Seroprevalence of *B. abortus* in cart horses from Foz do Iguaçu and correspondent testing of risk factors

***B. abortus* (2-mercaptoethanol)**			
		+/n	**(%)**
Age	>5	1/39	2.56
	≤5	0/22	0.00
Gender	Male	0/30	0.00
	Female	1/31	3.23
Abortion	Yes	1/3	33.33
	No	0/28	0.00
Graze	Yes	1/60	1.67
	No	0/1	0.00
Multispecies	Yes	1/48	2.08
grazing	No	0/13	0.00

+/n, Number of positive animals; %, soroprevalence

4.5 DISCUSSION

The present study resembles the only other study on the same population that has been conducted in Brazil, which evaluated infection by *Brucella abortus* in carthorses in Curitiba and São José dos Pinhais, Paraná. Out of the 123 samples subjected to the Rose Bengal test (RBT) and the slow agglutination test using 2-mercaptoethanol (2-ME) for confirmation of the results, 6.5% of the horses were positive for RBT and only one case was confirmed through 2-ME (ANTUNES et al, 2013).

Currently, several serological tests are available, but different authors have reported that nonspecific reactions commonly occur and that there is difficulty in standardizing significant titers of the serum (FERNANDES et al, 2014).

There is a report from Mexico in which out of 420 samples relating to drinking of water from a pond, the sample from one stallion showed positive results through the Rose Bengal test (RBT). However, but this result was considered to be a false positive after serological and epidemiological analyses were carried on in other horses under the same environmental conditions (GONZÁLEZ et al, 2006).

Nevertheless, some studies in different countries have reported seroprevalence of *Brucella abortus* antibodies in horses, thus corroborating our study in Foz do Iguaçu. The epidemiological importance of equine brucellosis in Costa Rica was more significant: seroprevalences of 6.5% and 21.7% were found in horse samples collected in 2014 and 2016, respectively. However, most of the samples were collected from horses that were used for sports, without correlation with other species (MORA et al, 2017).

Two studies were carried out in Nigeria. The first one presented seroprevalence of 11/75 samples from horses (14.7%) and the second did not find any positive horses. In both studies, the animals cohabited with several other species (EHIZIBOLO et al, 2011 and ARDO et al, 2016). Just like in Nigeria, a study in Eritrea found that there were no positive horses among a total of 82 samples. However, that study presented the first serological evidence of infection in camels with two positive samples out of 98 (3%) (OMER et al, 2000).

In Brazil, control and/or prophylaxis measures relating to brucellosis have focused on cattle and buffalos because of the higher prevalence of this disease in these species and the presence of official regulations from the National Program for Control and Eradication of Brucellosis and Tuberculosis (PNCEBT). On the other hand, there are no recommendations for specific disease control for equine brucellosis.

However, the presence of several animal species in urban areas, often coming from rural areas, gives rise to a need for updating the public health measures for brucellosis. These should, in the future, prioritize direct contact between people and animals (GODFROID et al, 2017).

4.6 CONCLUSION

Cohabitation with several species may pose a risk of contamination by brucellosis. Although the results from our study are insufficient to determine the epidemiological significance of such infection, research in this field needs to be continued.

In rural areas, horses should be evaluated as sentinel animals for the disease, with the aim of avoiding unexpected outbreaks on large farms.

The proximity of positive animals and people living under vulnerable conditions in the tri-border region, sometimes without basic sanitation, may pose a risk to public health.

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5. GENERAL CONCLUSIONS

*The present study shows that only three Secretariats of Health in Brazil initiated protocols for the control of human brucellosis: Santa Catarina, Paraná and Tocantins.

*Demonstrated seroprevalence of 4,83% for *Brucella ovis* antibodies in goats from Paraíba, there being no seroprevalence of antibodies to *Brucella abortus*, although the techniques for diagnosis of *Brucella abortus* are standardized only for cattle.

*Demonstrated the seroprevalence of 1,64% *Brucella abortus* antibodies in cart horses in Foz do Iguaçú, serving as an alert because these animals live in the urban area of the municipality cohabiting with people and other species.

*There are no efforts by the health authorities to control the disease in other animal species, since there is only the health program for cattle.

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7. SUPPLEMENTS

7.1 Protocol for release of animals for research

COMISSÃO DE ÉTICA NO USO DE ANIMAIS

NOTIFICAÇÃO

João Pessoa, 25 de setembro de 2014
CEUA Nº **3305/14**

Ilmo(a). **Rafael Felipe da Costa Vieira**
Departamento **Ciências Veterinárias - CCA - UFPB**

Orientando(a): **Rafael Felipe da Costa Vieira, (Mestrado)**

A Comissão de Ética no Uso de Animais do Centro de Biotecnologia da Universidade Federal da Paraíba em sua reunião ordinária de **25/09/2014** analisou e **APROVOU** a execução do projeto **Caracterização epidemiológica de patógenos transmitidos por carrapatos, Leishmania sp., Toxoplasma gondii e Neospora caninum em caprinos e equinos do Estado da Paraíba.**

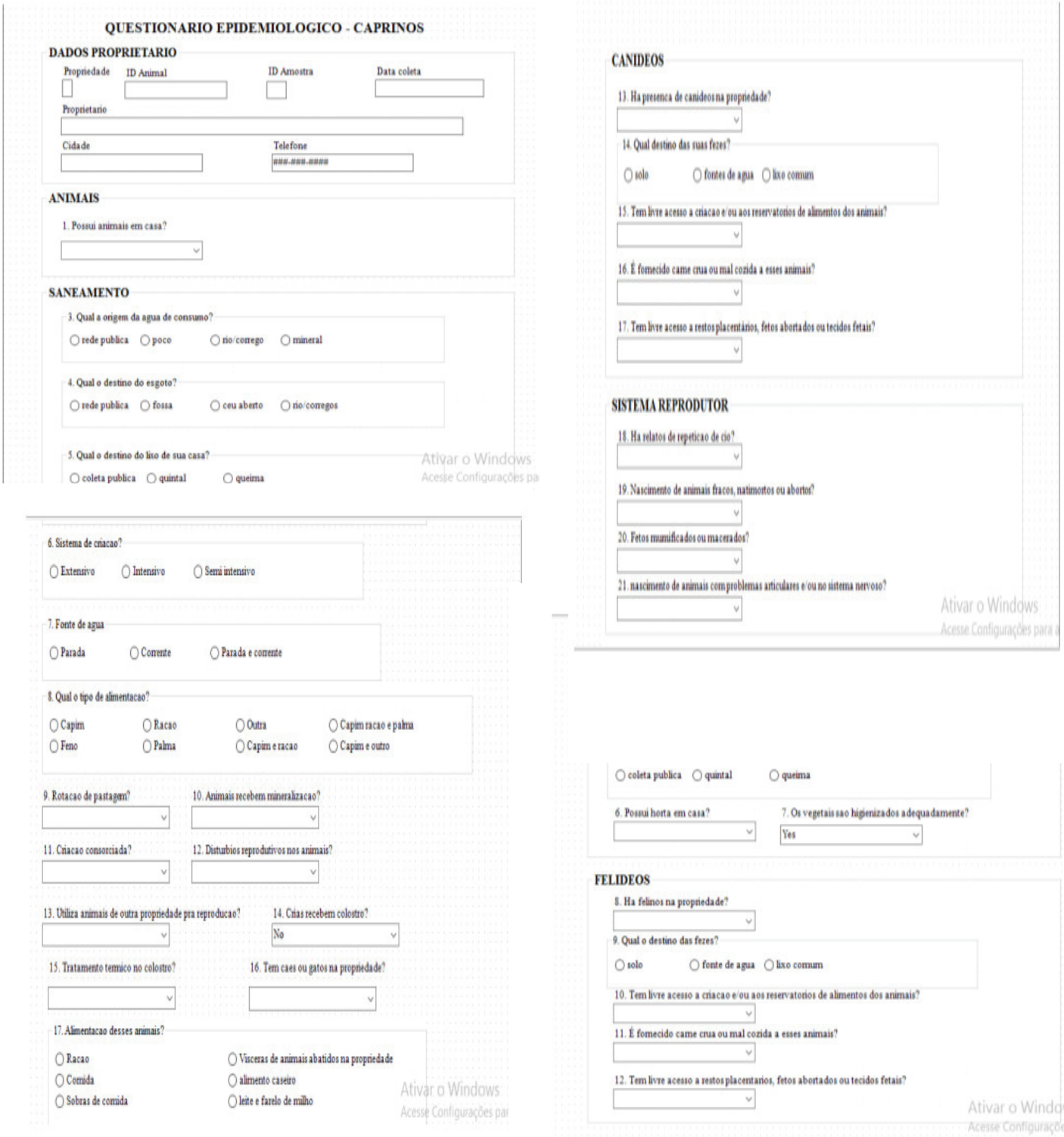
Com previsão de empregar **380 Caprinos, 380 Equídeos - Propriedades rurais**.

Para serem utilizados no período de **01/05/2014** a **31/07/2016**

Atenciosamente,

Prof. Dr. Luis Cezar Rodrigues
Presidente da Comissão de Ética no Uso de Animal do CBiotec/UFPB

7.2 Epidemiological questionnaire - goats



QUESTIONARIO EPIDEMIOLOGICO - CAPRINOS

DADOS PROPRIETARIO

Propriedade | ID Animal | ID Amostra | Data coleta

Proprietario

Cidade | Telefone ###-###-####

ANIMAIS

1. Possui animais em casa?

SANEAMENTO

3. Qual a origem da agua de consumo?
○ rede publica ○ poco ○ rio/corrego ○ mineral

4. Qual o destino do esgoto?
○ rede publica ○ fossa ○ ceu aberto ○ rio/corregos

5. Qual o destino do lixo de sua casa?
○ coleta publica ○ quintal ○ queima

6. Sistema de criacao?
○ Extensivo ○ Intensivo ○ Semi intensivo

7. Fonte de agua
○ Parada ○ Corrente ○ Parada e corrente

8. Qual o tipo de alimentacao?
○ Capim ○ Racao ○ Outra ○ Capim racao e palma
○ Feno ○ Palma ○ Capim e racao ○ Capim e outro

9. Rotacao de pastagem? | 10. Animais recebem mineralizacao?

11. Criacao consorciada? | 12. Disturbios reprodutivos nos animais?

13. Utiliza animais de outra propriedade pra reproducao? | 14. Crias recebem colostro? No

15. Tratamento termico no colostro? | 16. Tem caes ou gatos na propriedade?

17. Alimentacao desses animais?
○ Racao ○ Visceras de animais abatidos na propriedade
○ Comida ○ alimento caseiro
○ Sobras de comida ○ leite e farelo de milho

CANIDEOS

13. Ha presenca de canideos na propriedade?

14. Qual destino das suas fezes?
○ solo ○ fontes de agua ○ lixo comum

15. Tem livre acesso a criacao e/ou aos reservatorios de alimentos dos animais?

16. É fornecido carne crua ou mal cozida a esses animais?

17. Tem livre acesso a restos placentários, fetos abortados ou tecidos fetais?

SISTEMA REPRODUTOR

18. Ha relatos de repeticao de cio?

19. Nascimento de animais fracos, natimortos ou abortos?

20. Fetos mumificados ou macerados?

21. nascimento de animais com problemas articulares e/ou no sistema nervoso?

○ coleta publica ○ quintal ○ queima

6. Possui horta em casa? | 7. Os vegetais sao higienizados adequadamente? Yes

FELIDEOS

8. Ha felinos na propriedade?

9. Qual o destino das fezes?
○ solo ○ fonte de agua ○ lixo comum

10. Tem livre acesso a criacao e/ou aos reservatorios de alimentos dos animais?

11. É fornecido carne crua ou mal cozida a esses animais?

12. Tem livre acesso a restos placentarios, fetos abortados ou tecidos fetais?

7.3 Epidemiological questionnaire – cart horses

UFPR

FICHA EPIDEMIOLÓGICA - PROJETO CARROCEIROS

Nº da ficha _____

SOBRE OS VETORES

Já foi picado por carrapato?	( )sim	( )não
Fazem controle de carrapato?	( )sim	( )não
Qual produto?		
Há presença de carrapatos no animal?	( )sim	( )não
Qual a época do ano que aparecem?	( )primavera	( )inverno
	( )verão	( )ano todo
	( )outono	
Qual a frequência no controle de carrapatos?	( )semestral	( )anual
Existem capivaras na região?	( )sim	( )não

SOBRE OS EQUINOS

Convive com outros animais?	( ) cães ( ) gatos	( ) bovinos
	( )aves ( )capri/ovi	( )suínos
Houve óbito de algum cavalo em sua propriedade?	( )sim	( )não
Há quanto tempo?		
Algum dos animais apresentou quadro neurológico?	( )sim	( )não
Se sim, quais?		
Houve óbito de aves no local ou em locais próximos?	( )sim	( )não
Os animais foram medicados recentemente?	( )sim	( )não
Se sim, porque?		
Recebeu recentemente cavalos de outros locais?	( )sim	( )não
Se sim, de onde?		
Enviaram recentemente cavalos para outros locais?	( )sim	( )não
Se sim, para onde?	( )sim	( )não

SOBRE OS HUMANOS

Qual a origem da água de consumo?	( )rede pública	( )rio/córrego
	( )poço	
Qual o destino do esgoto?	( )rede pública	( )fossa
	( )céu aberto	( )rio/córregos
Qual o destino do lixo da sua casa?	( )coleta pública	( )quintal
	( )queima	
Possui horta em casa?	( )sim	( )não
Há histórico de meningite ou encefalite na família?	( )sim	( )não

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FICHA DE CADASTRO - PROJETO CARROCEIROS

Nº da ficha

PROPRIETÁRIO

Nome	
Telefone	
RG	
Endereço	
Bairro	
Escolaridade	
Quantas pessoas moram na casa?	
Quantas trabalham?	
Quantas crianças até 12 anos?	
Quantas com idade entre 12 e 18 anos?	
Qual a renda mensal aproximada?	
É dono do animal?	( )Sim ( )Não
Quantos cavalos possui?	
Qual o maior problema enfretado pelo senhor (a) no município?	

ANIMAL

Nome	
Raça	
Sexo	( )F ( )M
No caso de machos: (assinalar)	( )Inteiro ( )Castrado
No caso de fêmeas: (assinalar)	( )Prenhe ( )Não prenhe ( )Lactente
Fêmeas: Já abortou?	( )Sim ( )Não
Se sim, quando foi o aborto?	
O animal trabalha puxando carroça?	( )Sim ( )Não
Há mais de uma animal que reveza puxando a carroça?	( ) Sim ( )Não
Que horas sai de casa?	
Que horas volta pra casa?	
Animal já foi vermifugado?	( ) Sim ( ) Não
Animal já foi vacinado?	( ) Sim ( ) Não
Durante o trabalho, o animal se alimenta?	( ) Sim ( ) Não
Durante o trabalho, o animal bebe água?	( ) Sim ( ) Não
Se sim, quanto de água é fornecido?	
Alimentação (assinalar)	( ) Pasto
	( ) Farelo de trigo
	( ) Milho
	( ) Ração
	( ) Sal
	Outros (especificar):
Casqueamento	( ) Sim ( ) Não
O animal já teve sangramento nasal?	( ) Sim ( ) Não
Se sim, durante o trabalho?	( ) Sim ( ) Não

8. VITA

Meila Bastos de Almeida is a veterinarian at the Federal University of Santa Maria (UFSM). Since 2009 at the Institute of Technology of Paraná (Tecpar) with production of veterinary immunobiological and is currently responsible for the center of experimental models of Tecpar.